6 # CHAPTER 6 Cell Signaling: Cytokines and Their Receptors

6.1 ## KEY POINTS

- The immune responses develop as a result of interactions between different cell populations.
- Cells interact by secreting signaling molecules such as cytokines and hormones.
- These signaling molecules bind to specific receptors on target cells.
- When signaling molecules bind to these receptors they cause a cell to alter its behavior through a process called signal transduction. As a result transcription factors are generated. These transcription factors then activate selected genes.
- As a result of gene transcription, cells alter their behavior and secrete new cytokines or other signaling molecules.

The immune system functions through many different cell types sending and receiving messages that are delivered in the form of chemical signals. Molecules secreted by one cell are carried to another, where they bind to receptors on the target cell surface. By receiving signals through their appropriate receptors, the target cells can be stimulated to behave in an appropriate manner. They may be told to divide or stop dividing; they may be stimulated to secrete their own signaling molecules; they may be told to commit suicide. Each cell lives in an environment where it is exposed to many different signaling molecules at any one time. The target cell must integrate these signals in some way and respond appropriately. In this chapter we review the signaling molecules secreted by cells, the receptors that receive these signals, and the way in which the received signals are interpreted by the receiving cell. It should be pointed out that other body systems receive many messages by way of the nervous system. While nerves undoubtedly do connect with cells of the immune system (see Chapter 17) and regulate some aspects of immunity, this appears to be a relatively unimportant route compared to signaling through soluble mediators.

The cells of the immune system secrete several hundred different proteins that regulate the immune responses by communicating among cells. These proteins are called cytokines (Box 6-1). Cytokines differ from conventional hormones in several important respects. For example, unlike classical hormones, which tend to affect a single target, cytokines affect many different cell types. Second, immune system cells rarely secrete a single cytokine at a time. For instance, macrophages secrete at least five interleukins (interleukin-1 [IL-1], IL-6, IL-12, IL-18) as well as tumor necrosis factor-α (TNF-α). Third, cytokines are “redundant” in their biological activities in that many different cytokines have similar effects. For example, IL-1, TNF-α, TNF-β, IL-6, high mobility group box protein-1 (HMGB1), and the chemokine CCL3 all act on the brain to cause fever. This complexity has given rise to the concept of a cytokine network, a web of signals transmitted among all the cell types of the immune system mediated by very complex mixtures of cytokines.

6.2 ## Box 6-1 Properties of Cytokines

- Short-lived proteins
- Highly diverse structures and receptors
- Can act locally and/or systemically

- Pleiotropic: affect many different cells
- Redundant: exhibit biologically overlapping functions
- Carefully regulated
- Toxic in high doses

6.3

Box 6-2 Cytokine Nomenclature

The naming of cytokines is the responsibility of the Interleukin Nomenclature Subcommittee of the International Union of Immunological Societies. This naming has been based on the each cytokine's origin and structure, as well as the demonstration of functional effects on leukocytes. Unfortunately, the gene nomenclature committee of the Human Genome Organization has recently assigned interleukin names to molecules based only on sequence similarity to other interleukins. The result has been a rash of duplications and mislabelings. For example, several different proteins have been called interleukin-25 (IL-25). This has caused such confusion that the name IL-25 is no longer in use. The number of "interleukins" has grown rapidly, while their biological significance remains quite unclear.

6.4

CYTOKINE NOMENCLATURE

The nomenclature of the cytokines is not based on any systematic relationship among these proteins. Many were originally named after their cell of origin or the bioassay used to identify them (Box 6-2).

The interleukins are cytokines that mediate signaling between lymphocytes and other leukocytes. They are numbered sequentially in the order of their discovery. Because their definition is so broad, the interleukins are a heterogeneous mixture of proteins with little in common except their name. As of 2007, 34 different interleukins have been described. As might be expected, we know a lot about some of these molecules while we know very little about others. Likewise some are critical to a successful immune response while others appear to be much less important.

The interferons are cytokines produced in response to virus infection or immune stimulation. Their name is derived from the fact that they interfere with viral RNA and protein synthesis and so block viral replication. There are two major types of interferon. The most important type I interferons are interferon-α (IFN-α) and interferon-β (IFN-β). There is a single type II interferon, called interferon-γ (IFN-γ). Type I interferons are primarily antiviral with a secondary immunoregulatory role. For type II interferons such as IFN-γ, the reverse is the case. Many type I interferons also play an important role in the maintenance of pregnancy.

TNFs are cytokines secreted by macrophages and T cells. As their name suggests, they can kill tumor cells, although this is not their primary function. Thus TNF-α is the key mediator of acute inflammation. The TNFs belong to a family of related cytokines, the TNF superfamily, which is involved in immune regulation and inflammation. Other important members of the TNF superfamily include CD178 (also called CD95L or fas ligand; see Chapter 16) and CD154 (CD40 ligand).

Many cytokines serve as growth factors (or colony-stimulating factors) and control leukocyte production by regulating stem cell activities. They thereby ensure that the body is supplied with sufficient cells to defend itself.

Chemokines are a family of at least 50 small proteins that play an important role in leukocyte circulation and migration, especially in inflammation. They are chemotactic factors and leukocyte activators. A typical example of a chemokine is CXCL8 (also known as IL-8). Chemokines are described in detail in Chapter 2.

6.5 CYTOKINE FUNCTIONS

Cytokines are produced in response to many different stimuli. The most important of these stimuli are antigens acting through the T cell or B cell antigen receptors, antigen-antibody complexes acting through antibody receptors *(FcRs)*, and pathogen-associated molecular patterns *(PAMPs)* such as lipopolysaccharides acting through toll-like receptors *(TLRs)* (Figure 6-1).

Cytokines act on many different cellular targets. They may, for example, bind to receptors on the cell that produced them and thus have an autocrine effect. Alternatively, they may bind only to receptors on nearby cells (this is called a paracrine effect). Some cytokines may spread throughout the body, affecting target cells in distant locations and thus having an endocrine effect (Figure 6-2).

When cytokines bind to receptors on target cells, they affect cell behavior. They may induce the target cell to divide or differentiate, or they may stimulate the production of new proteins. Alternatively they may inhibit these effects by preventing division, dif

71

72

FIGURE 6-1 Three of the most important reactions that trigger cytokine release are the combination of antigens with their receptors on T and B cells; the combination of pathogen-associated molecular patterns (PAMPs) with toll-like receptors (TLR); and the combination of antibodies with Fc receptors (FcR).

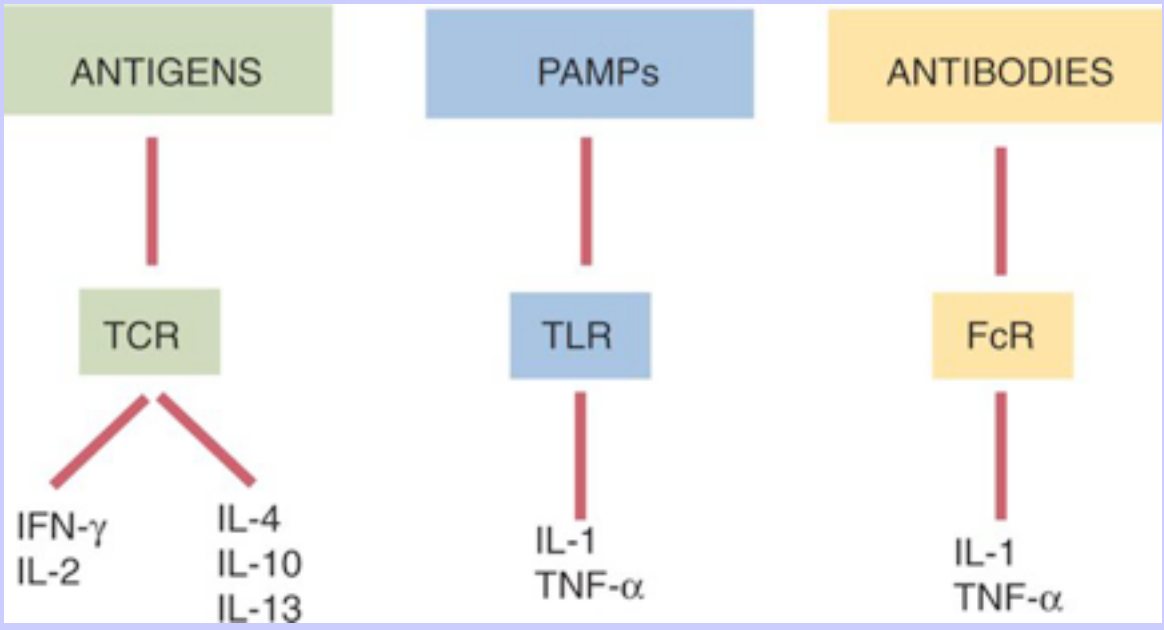
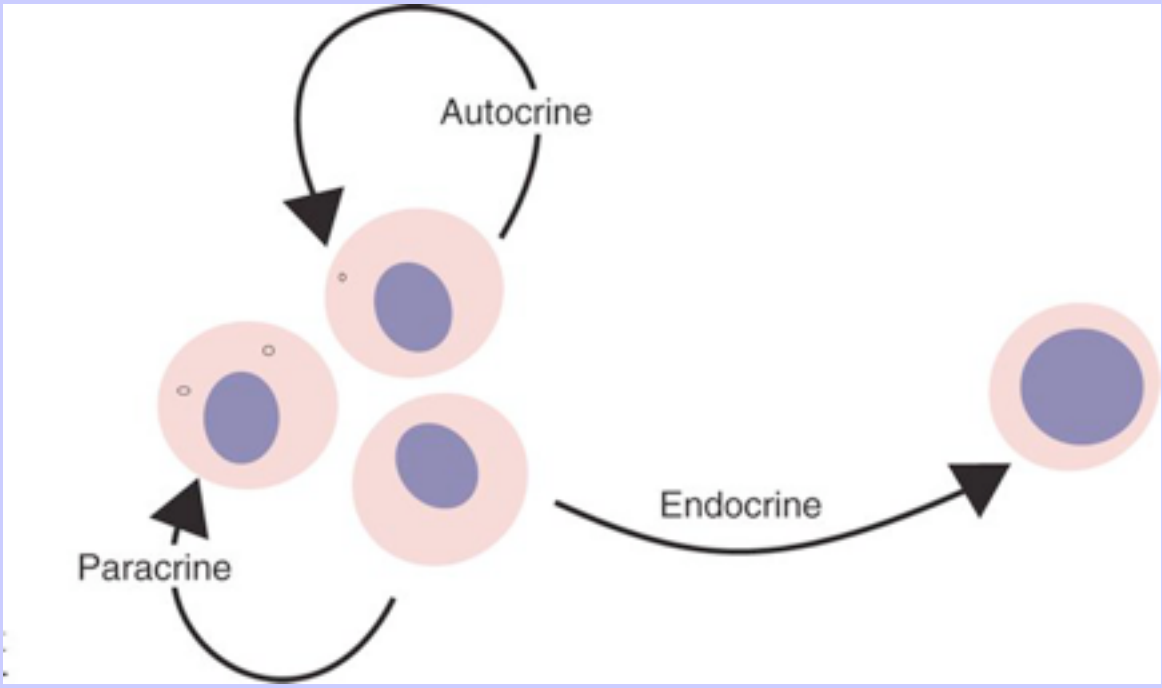


FIGURE 6-2 The distinction among autocrine, paracrine, and endocrine effects. Cytokines differ from hormones in that most of their effects are autocrine or paracrine, whereas hormones act on distant cells in an endocrine fashion.



ferentiation, or new protein synthesis. Most cytokines act on many different target cell types, perhaps inducing different responses in each one, a feature that is called pleiotropy. Conversely, many different cytokines may act on a single target, a feature known as redundancy. For example, IL-3, IL-4, IL-5, and IL-6 all affect B cell function. Some cytokines work best when paired with other cytokines in a process called synergy. For example, the combination of IL-4 and IL-5 stimulates B cells to make immunoglobulin E (IgE) and hence triggers an allergic response. Synergy can also occur in sequence when, for example, one cytokine induces the target cell to express the receptor for another cytokine. Finally, some cytokines have opposing effects and so may antagonize the effects of others. The best example of this is the mutual antagonism of IL-4 and IFN-γ.

Table 6-1 A Molecular Classification of the Cytokines

Structural Families	Structure	Examples
Group 1	Four α helix bundle	IL-2, –3, –4, –5, –6, –7, –9, –11, –13, –15, –21, –23, –30; GM-CSF, erythropoietin, G-CSF, prolactin, leptin
	Interferon subfamily	IFN-α/β, IFN-γ
	Interleukin-10 subfamily	IL-10, –19, –20, –22, –24, –26
Group 2	Beta sheets	TNFs, TGF-β, IL-1α, IL-1β, IL-18
Group 3	α Helices and β sheets	Chemokines
Group 4	Mixed motifs	IL-12
Ungrouped	Unique structures	IL-17A-F, IL-14, IL-16

6.6 CYTOKINE STRUCTURE

Cytokines are proteins with diverse structures. They can, however, be classified into several structural families (Table 6-1).

The largest family, the group 1 cytokines (or hematopoietins), consists of four α-helices bundled together. They include many different interleukins as well as growth hormone and leptin. Within the group 1 cytokines are two major subfamilies of related proteins, the interferon subfamily and the IL-10 subfamily.

In contrast, group 2 cytokines consist of long chain β-sheet structures. They include the TNFs, the IL-1 family, and TGF-β. Group 3 cytokines are small proteins with both α-helices and β-sheets. These include the chemokines and related molecules (see Chapter 2). Group 4 cytokines are constructed using domains with mixtures of different structural motifs and include IL-12. The IL-17 family, IL-14, and IL-16 are structurally unique proteins and do not belong to any of these major families.

Patterns may also be seen in the biological activities of these cytokines. Thus group 1 cytokines are all involved in immune regulation or stem cell regulation. Group 2 cytokines are mainly involved in the growth and regulation of cells, cell death, and inflammation. Group 3 cytokines are involved in inflammation. The activities of the group 4 cytokines

FIGURE 6-3 The structure of a cytokine receptor. In this case the interleukin-2 *(IL-2)* receptor complex. The complete trimer serves as a high-affinity receptor while the β–γ dimer serves as a low-affinity receptor.

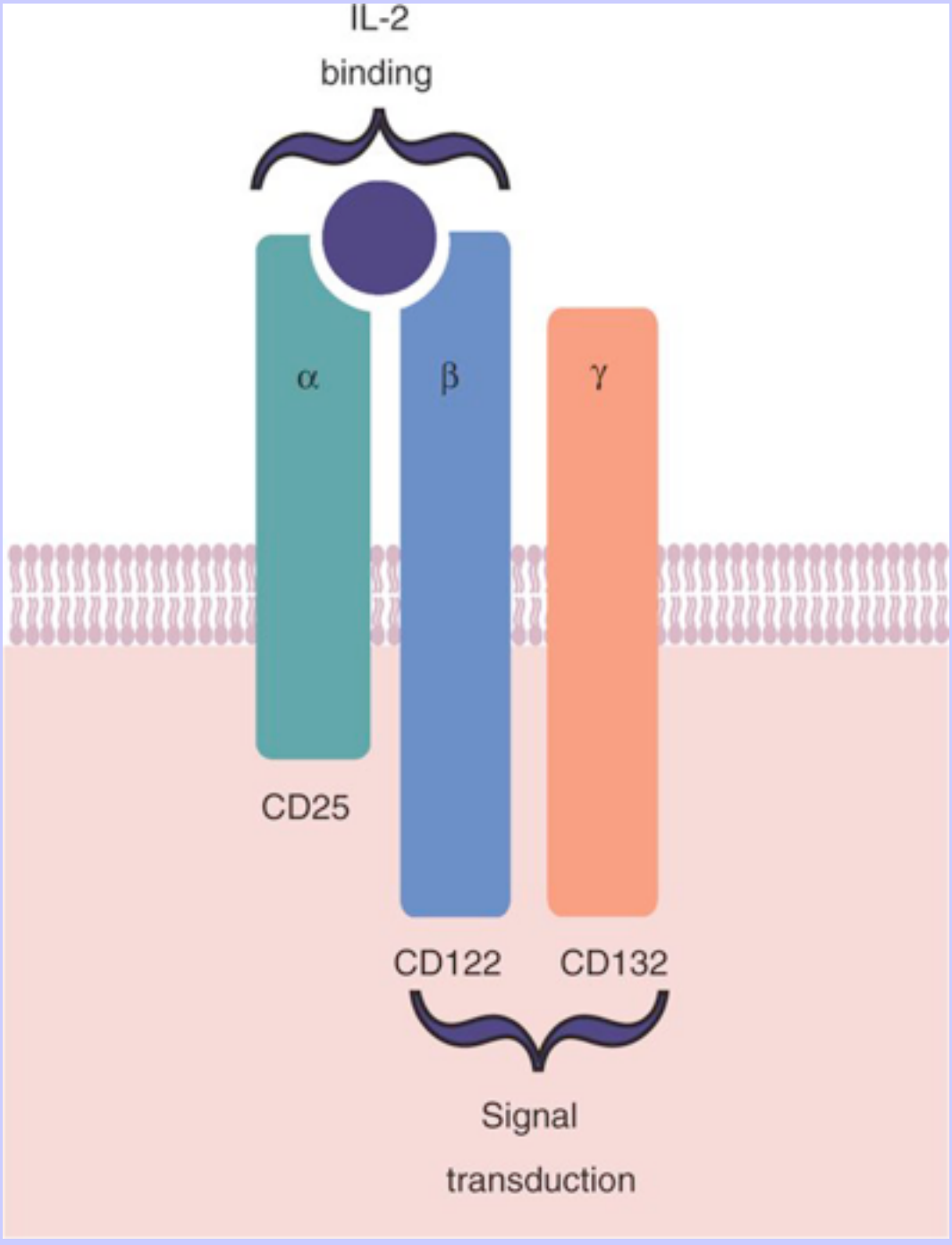
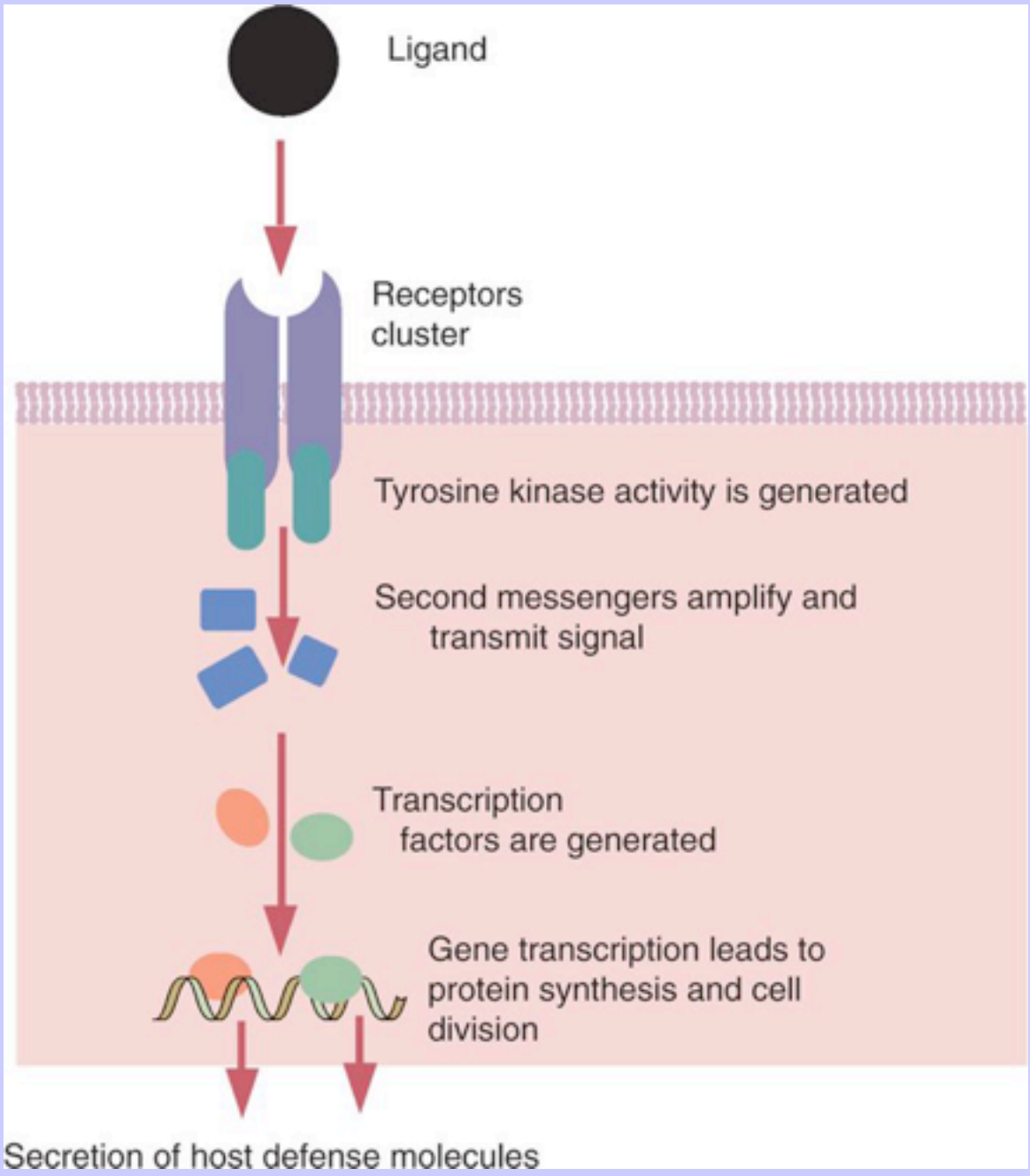


FIGURE 6-4 Generic view of signal transduction involving the activation of tyrosine kinases. Although receptor signaling varies in its details, the overall process of signal transduction has some consistent features shown here.



depend on their subcomponents. For example, IL-12 is formed by a combination of a group 1 structure with a stem cell receptor, but it acts like a group 1 cytokine.

6.7 CYTOKINE RECEPTORS

Cytokines act through cell surface receptors. These receptors consist of at least two functional units, one for ligand binding and one for signal transduction (Figure 6-3). These may or may not be on the same protein. Cytokine receptors can also be classified based on their structure.

One class, the channel-linked receptors, serves as transmitter-gated ion channels. Thus the receptor itself is a channel, and binding of its agonist opens that channel allowing ions to pass through it. Channel-linked receptors are found in inflammatory and immune cells, but their roles are unclear. They do not serve as cytokine receptors.

A second class of receptor consists of proteins that also act as tyrosine kinases (TKs) (Figure 6-4). These are typically growth factor and cytokine receptors. In these cases binding of the ligand to two adjacent receptors forms an active dimer. The receptor site, the membrane-spanning region, and the effector enzyme are usually separate domains of a single protein. Thus when the ligand binds to the extracellular domain and the receptors dimerize, the two TKs are brought together and activate each other. These kinases phosphorylate tyrosine residues on other proteins or even the receptor itself (autophosphorylation). Since many of these other proteins are also TKs, it also converts them to an active state. In this way a cascade of expanding phosphorylations develops within the cell (Figure 6-5). The phosphorylation triggers changes in cellular activities. Many cytokines and other immunological signals operate through this type of receptor (especially through protein kinases of the Src family). A related class of receptor consists of proteins that are not themselves TKs but can activate TKs associated with the receptors. This type of receptor is also widely employed in the cells of the immune system. Examples of TK-linked receptors include the T cell antigen receptor (TCR) and the B cell antigen receptor. Some of these TKs may transfer their phosphate groups to transcription factors within the nucleus and activate them. Others act indirectly through the production of second messengers.

A large class of receptors is associated with membrane-bound guanosine triphosphate (GTP)–binding

74

FIGURE 6-5 The key to cellular activation is the phosphorylation of tyrosine residues by the actions of a tyrosine kinase. For example, phosphorylation of tyrosine by a protein kinase results in phospholipase activation, which leads eventually to cell activation.

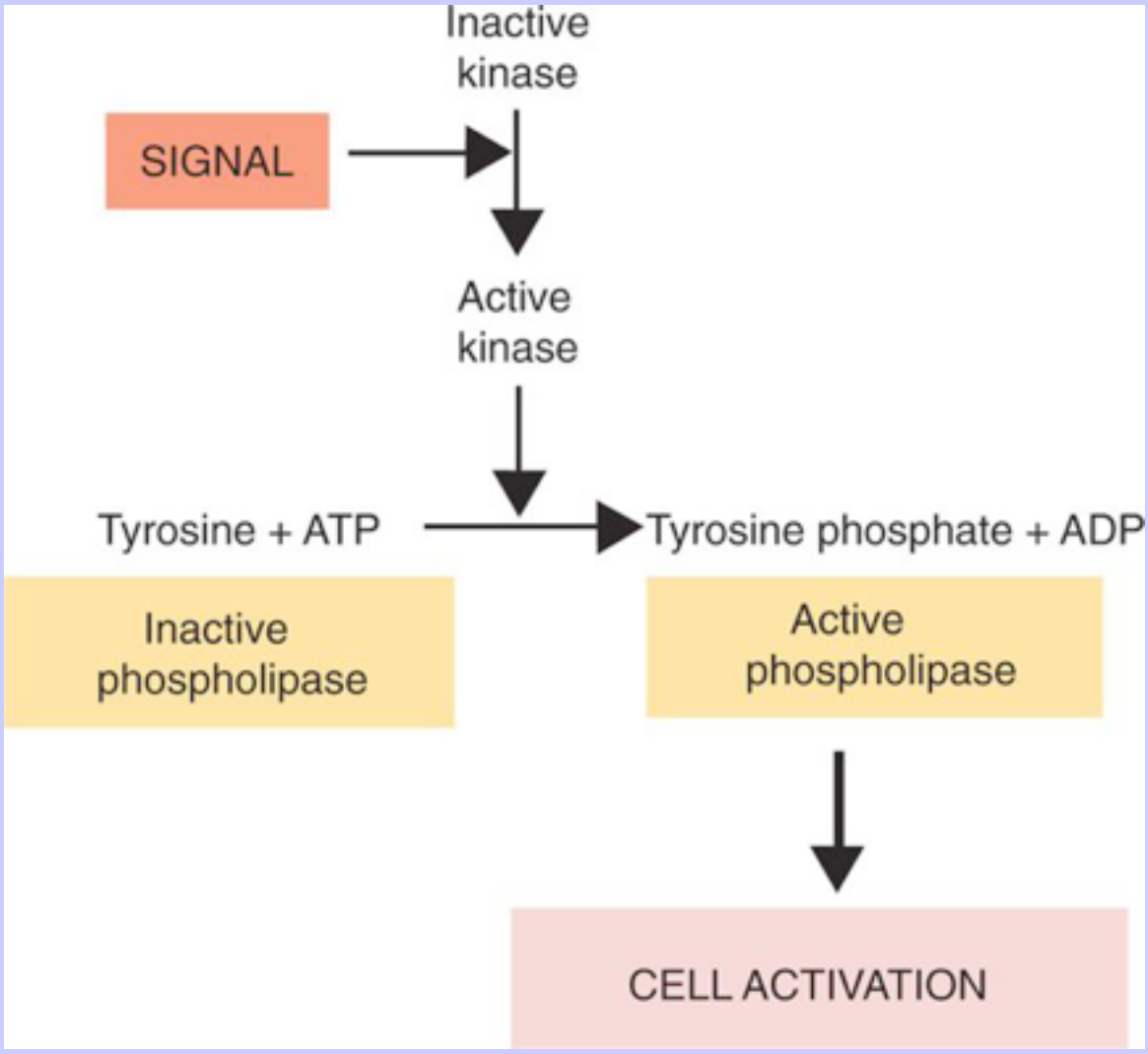
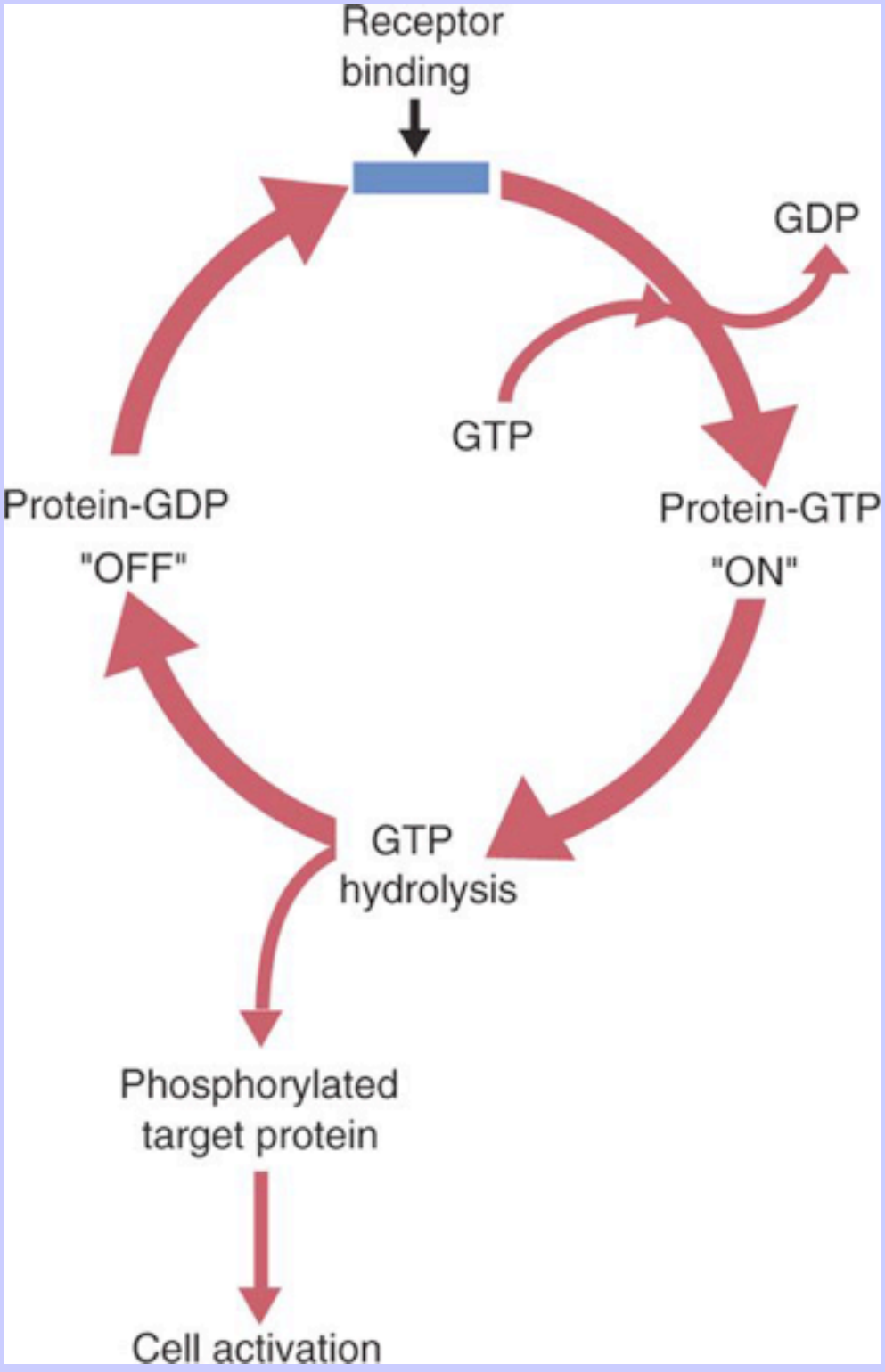


FIGURE 6-6 G proteins act as a signaling switch to turn cell functions on and off.



proteins, called G proteins. G proteins act as chemical switches and so control many different cellular processes. When inactive, they bind guanosine diphosphate (GDP). When active, they bind GTP. Thus when these receptors bind their ligand, a change in the receptor/G-protein complex results in a loss of GDP and a gain of GTP (Figure 6-6). The activated G protein then activates other substrates. The GTP is rapidly hydrolyzed to GDP so that the G protein is then turned off. The targets of G proteins can include ion channels, enzymes such as adenylate cyclase, phospholipase C, and some protein kinases. When activated by a G protein, phospholipase C splits the membrane-bound lipid phosphatidylinositol 4,5-bisphosphate (PIP_2) into two messenger molecules, inositol trisphosphate and diacylglycerol (Figure 6-7). Inositol trisphosphate binds to intracellular receptors releasing Ca^{2+} from internal stores and so increases the concentration of intracellular Ca^{2+}. These calcium ions can activate many different proteins. The diacylglycerol remains in the plasma membrane and along with calcium activates an enzyme called protein kinase C. The only immunologic receptors that employ G proteins are the receptors for chemokines—C5a and platelet-activating factor.

A fourth class of receptor activates a neutral sphingomyelinase that then hydrolyzes sphingomyelin in the cell membrane to ceramide. The ceramide then stimulates a ceramide-activated serine-threonine protein kinase that phosphorylates cellular proteins. This mechanism of signal transduction is used by the receptors for IL-1 and IFN-α.

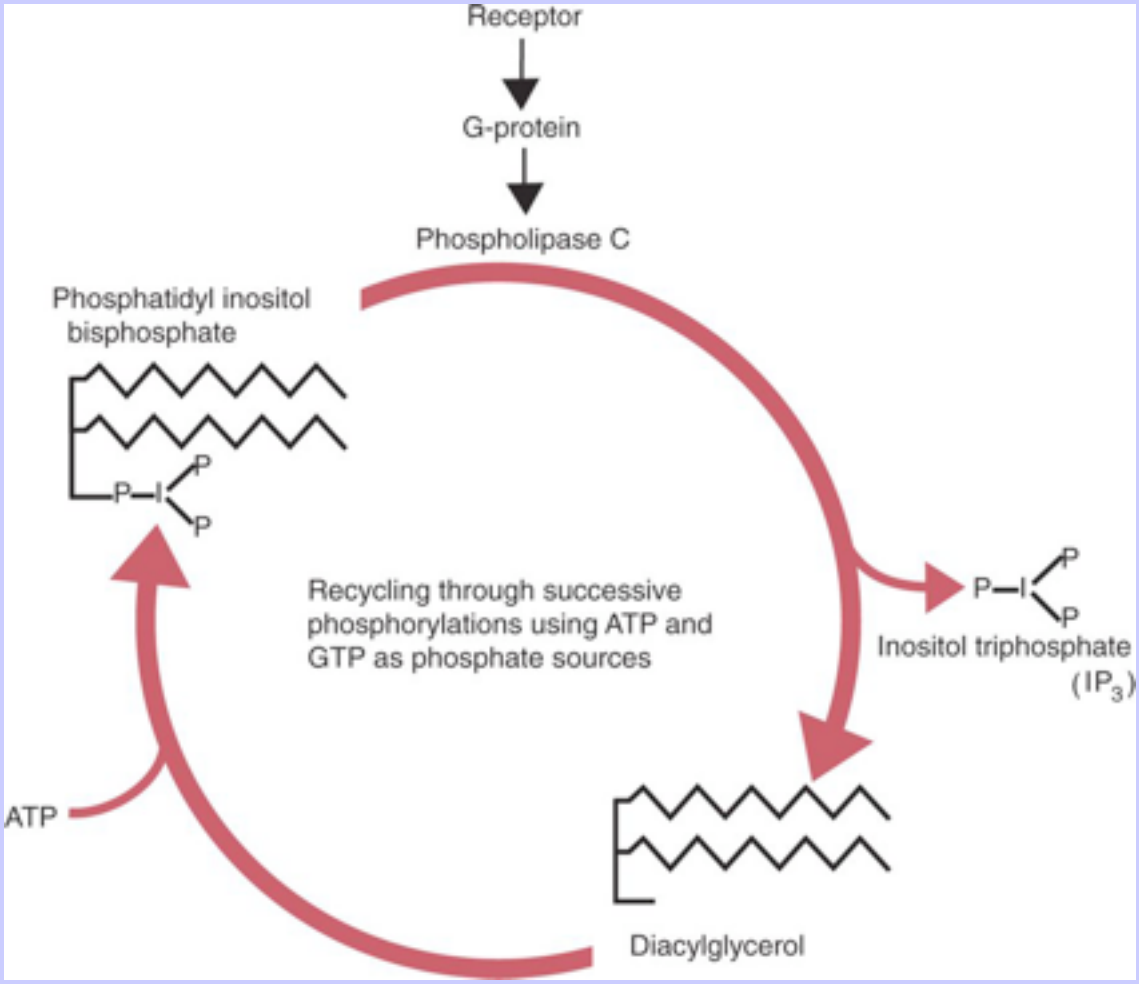
6.7.1 Functional Families

In general, cytokines use receptors that act through TKs. However, within this class, one can identify related families of receptors. For example, the IL-1/TLR receptor family consists of receptors that participate in host responses to injury and infection. They include the receptors for IL-1 and IL-18, as well as the TLRs. The family can be split into the molecules that are IL-1R–like (IL-1R1, IL-18R) and the molecules that are toll-like (TLR). Ligation of these receptors triggers activation of the transcription factor nuclear factor kappa-B (NF-κB).

Another family, the group I cytokine receptor family, includes the receptors for IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-12, IL-13, IL-15, IL-21, granulocyte colony-stimulating factor R (G-CSFR), granulocyte-macrophage colony-stimulating factor R (GM-CSFR), IFN-α/βR, IFN-γR, and IL-10R (Figure 6-8). It also includes the common β chain of IL-3, IL-5, and GM-CSF receptors and the common γ chain of IL-2, IL-4, IL-7, IL-9, and IL-15 receptors. These receptor chains dimerize in the presence of the ligand and form complexes with a separate kinase called a Janus kinase (JAK). JAK phosphorylates a cytosolic protein called signal transducer and activator of transcription (STAT). STAT, in turn, dimerizes to form an active transcription factor.

Other group I cytokine receptors bind interferons and cytokines related to IL-10 (IL-19, –20, –22, –24, –26)

FIGURE 6-7 Activation of cell membrane phospholipase C generates inositol triphosphate and diacylgycerol. These two molecules are messengers that initiate cell activation.



and two IFN-λ molecules (IL-28 and IL-29). These proteins form heterodimers in the presence of the ligand and also signal through the JAK-STAT pathway, leading to cytokine-specific responses. They differ from the class I receptor family in some of their conserved amino acid sequences.

6.8 CYTOKINE REGULATION

Cytokine signaling is regulated in three major ways: by changes in receptor expression, by specific binding proteins, and by cytokines that exert opposite effects. For example, IL-2 receptor expression largely determines the response of T cells to IL-2. T cells express few receptors for IL-2 when resting but many more once activated. In contrast, the activities of IL-1 are regulated by a receptor antagonist called IL-1RA. IL-1RA is an inactive form of IL-1 that binds to the IL-1 receptor but does not stimulate signal transduction. It therefore blocks the activities of active IL-1 (Figure 6-9). Some cytokines may bind to soluble receptors in body fluids. Examples include the soluble receptors for IL-1,

IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, TNF-α, and M-CSF. In most cases these soluble receptors compete for cytokine binding with cell surface receptors and hence inhibit their activities. Cytokines such as IL-1, IL-12, and TGF-β may bind to glycosaminoglycans such as heparin or CD44 in connective tissue, where they form a reservoir of readily available molecules. Perhaps the most important way by which cytokine function is regulated is through the opposing effects of different cytokines. For example, IL-4 stimulates B cells to switch to IgE production, whereas IFN-γ suppresses IgE production (see Chapter 25).

It is also important to bear in mind that at any given time, a single cell is receiving signals from multiple cytokine receptors. It must somehow integrate these multiple signals to produce a coherent response.

6.9 SIGNAL TRANSDUCTION

Cytokines and other molecules act as ligands for their cell surface receptors. Once the ligand binds its receptor, the receptor transmits a signal to the cell to modify its behavior. This conversion of an extracellular signal into a series of intracellular events is called signal transduction. The key components of signal transduction include binding of an agonist to a receptor, activation of a transducer protein by the receptor, secondary activation of other enzymes, generation of new transcription factors, and gene activation leading to altered cell behavior. Because cell signaling must be fast and precise, it is best accomplished by enzyme cascades. Since enzymes can produce or modify a large number

FIGURE 6-8 The major types of cytokine receptors. Growth factor receptors associate in the presence of their ligand to form a dimer. This brings the tyrosine kinases on the cytoplasmic domains close together. The enzymes are then activated by cross-phosphorylation. Group I cytokine receptors, such as those for interleukin-2 *(IL-2)*, associate in the presence of the ligand to form oligomers and form complexes with Janus kinases *(JAK)*. When brought together, the JAKs are activated and in turn activate signal transducer and activator of transcription (STAT) proteins. The STAT proteins dissociate and activate transcription factors. Cytokines such as the interferons and IL-10 bind receptors that have a similar mode of action to type I receptors. They differ, however, in their conserved sequences.

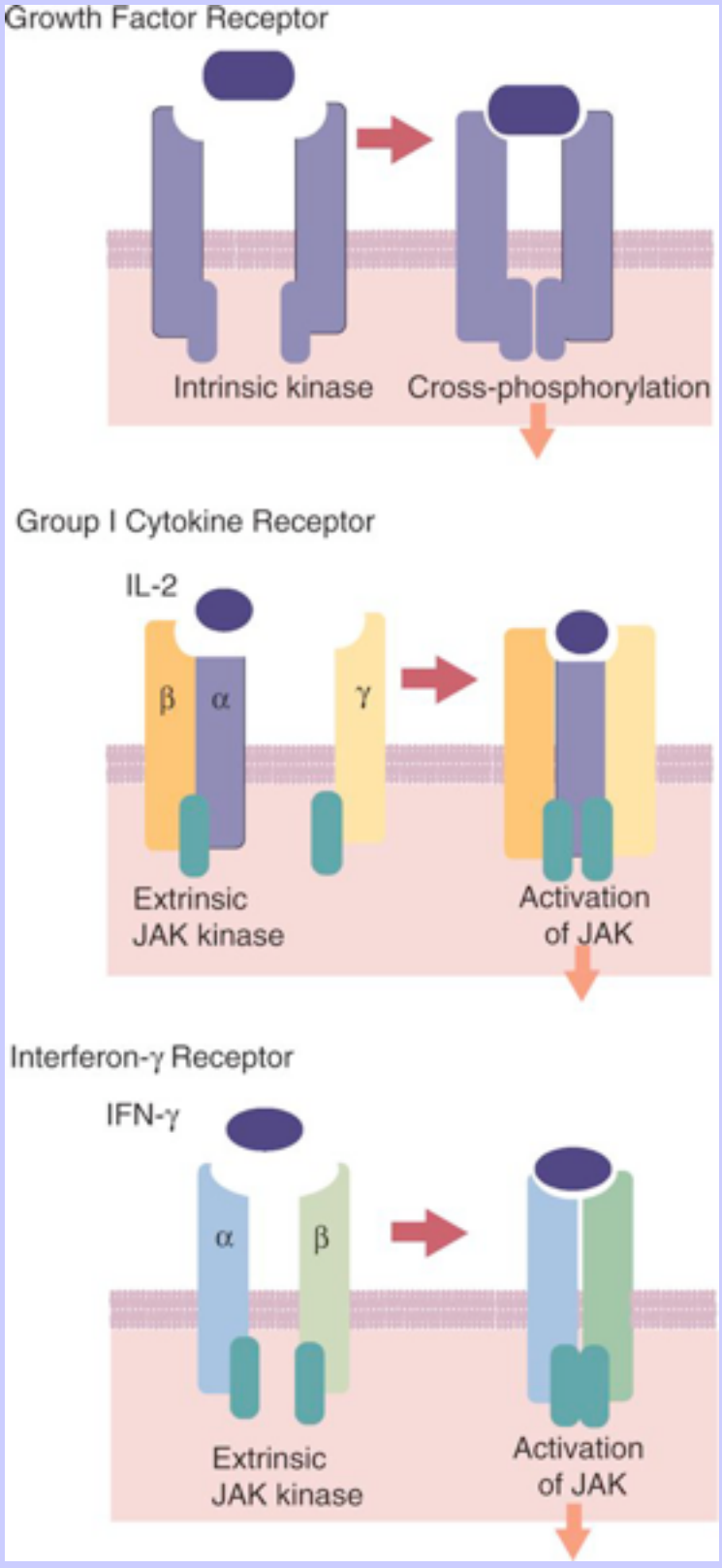
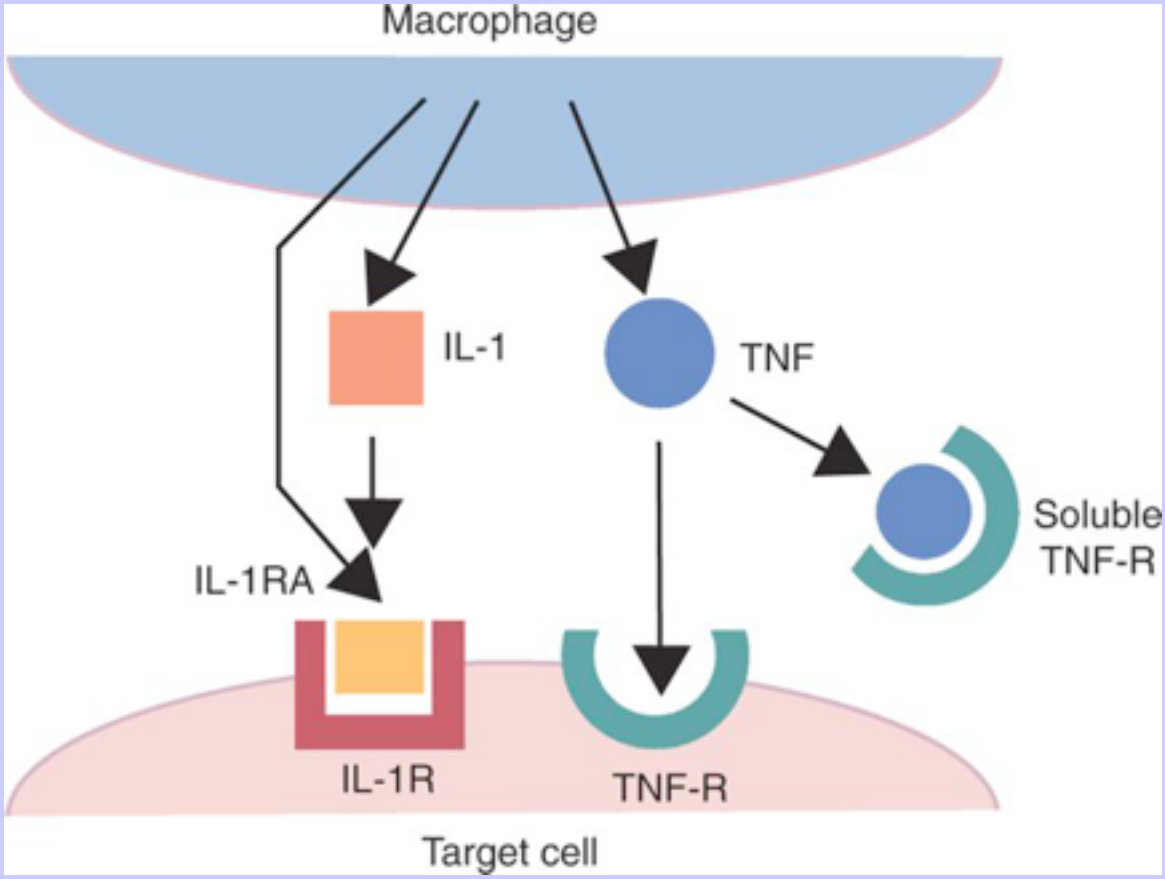

Growth Factor Receptor
Intrinsic kinase
Cross-phosphorylation
Group I Cytokine Receptor
IL-2
β
α
γ
Extrinsic
JAK kinase
Activation
of JAK
Interferon-γ Receptor
IFN-γ
α
β
Extrinsic
JAK kinase
Activation
of JAK

FIGURE 6-9 The control of cytokine activities as exemplified by interleukin-1 *(IL-1)* and tumor necrosis factor-α (TNF-α). IL-1 activity is regulated by the presence of IL-1RA, an inert isoform that binds and blocks the IL-1R, preventing signal transduction. Soluble TNF-αR, in contrast, competes for TNF-α with the cell-membrane receptor.



of molecules very rapidly, a pathway that involves the use of several enzymes in sequence can amplify responses very rapidly.

Central to most receptor signaling is the use of protein phosphorylation. Phosphorylation is a form of reversible modification of proteins. All signal transduction systems involve the use of a high-energy compound (e.g., GTP) to modify a protein and send a signal to a cell. Cell growth, cell division, and other critical processes are all regulated by protein phosphorylation. Protein kinases enzymatically phosphorylate the amino acids serine, threonine, and tyrosine.

$$\text{Protein} + \text{ATP} \xrightarrow{\text{Protein kinase}} \text{protein} - \text{P} + \text{ADP}$$

In some proteins only one amino acid is phosphorylated; in others, multiple amino acids are phosphorylated. Phosphorylated and nonphosphorylated proteins have different functional properties. For example, the

phosphorylation of serine or threonine activates some enzymes, whereas dephosphorylation has the opposite effect. Phosphorylation of the three key amino acids (serine, threonine, and tyrosine) plays a critical role in the regulation of many cellular functions. When phosphorylated proteins are examined, about 90% of the phosphate is attached to serine and about 10% to threonine. Only about 1/2000 of the phosphate is linked to tyrosine. Thus tyrosine phosphorylation is a rare event despite its being a key mechanism in almost all the signal transduction pathways described in this book.

6.9.1 Signal Transduction Pathways

While there are many different pathways of signal transduction, three play key roles in the immune system. These involve the generation of the transcription factors NF-κB, nuclear factor of activated T cells (NF-AT), and JAK/STAT.

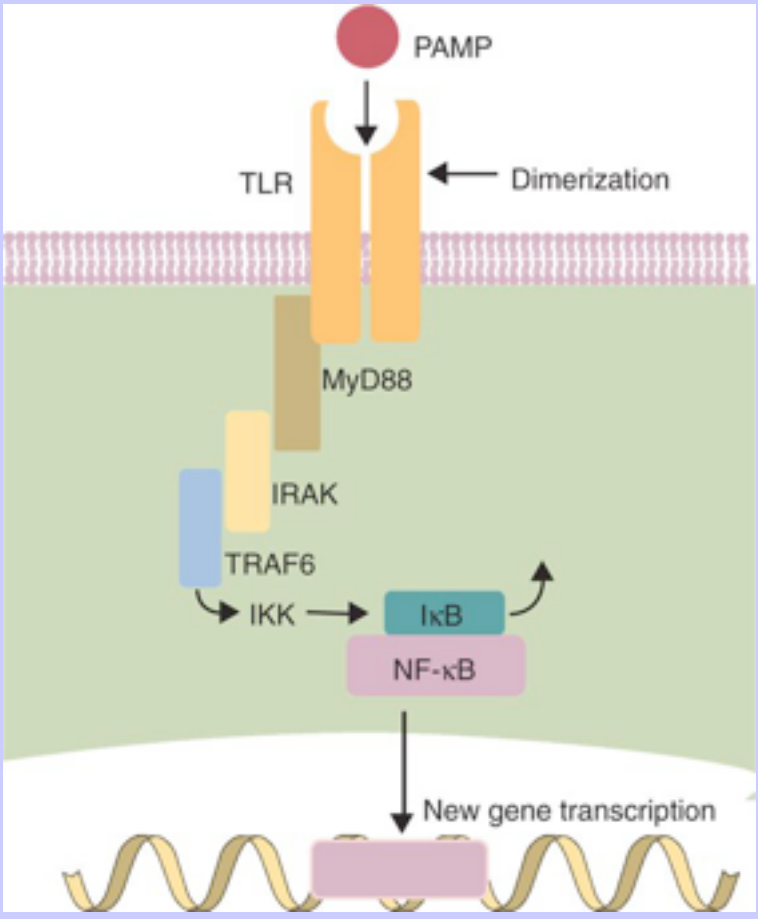
6.9.1.1 The NF-κB Pathway

The term *NF-κB* refers to a family of five transcription factors that play a central role in inflammation and

76

77

FIGURE 6-10 Signal transduction mediated through toll-like receptors (TLR) generates nuclear factor kappa-B (NF-κB).



immunity. All members of the family can form homodimers and heterodimers with one another. NF-κB exists in the cell cytoplasm in an inactive form through association with the IkB proteins. The IkB proteins inhibit NF-κB activity by masking its nuclear binding site so that in a resting cell, NF-κB cannot move to the nucleus or activate genes. More than 150 different stimuli have been shown to activate NF-κB, and more than 150 genes have been shown to be expressed on NF-κB activation. Three major NF-κB activation pathways have been identified. The "classical" pathway is involved in proinflammatory signaling. It is activated by inflammatory cytokines (IL-1 and TNF-α), TLRs, and antigen receptors and is essential for innate immunity. The signals induced by these stimuli converge on a central regulator of NF-κB, the IKK (IkB kinase) complex. The IKK complex consists of multiple subunits with kinase activity. When a cell is stimulated, the IKK complex phosphorylates IkB. As a result, IkB dissociates from the NF-κB. The newly released IkB becomes ubiquinated and is destroyed by proteasomes. This liberates the NF-κB that can now enter the nucleus, where it binds and activates kB motif–containing promoters on DNA. This results in activation of many genes including the cytokines IL-1β, IL-6, IL-18, IL-33, TNF-α, GM-CSF, and IL-4. NF-κB triggers activation of several different chemokine genes, proangiogenic factors, adhesion molecules such as intercellular adhesion molecule-1, antiapoptotic proteins, inducible enzymes (e.g., inducible nitric oxide synthase, cyclooxygenase-2), and also of more IkB (which ultimately downregulates NF-κB activation). Molecules or organisms that block the destruction of IkB will have antiinflammatory and immunosuppressive effects. Thus corticosteroids stimulate the production of excess IkB whereas some bacteria can block its degradation. Either way, the activation of cells and the development of inflammation and immune responses will be blocked. A second NF-κB pathway (sometimes called the alternative pathway) is triggered by a subset of TNF receptors. This pathway is essential for lymphocyte development and activation. The third NF-κB pathway is activated by DNA-damaging drugs and ultraviolet light. It does not involve IKK activation.

An example of the use of the NF-κB pathway is seen with TLR responses in macrophages (Figure 6-10). Occupation of a TLR by a PAMP or by HMGB1 causes the receptor to dimerize and change its shape. As a result, it binds several adaptor molecules of which one, MyD88 (myeloid differentiation primary response gene 88), is the most important. When MyD88 complexes with the TLR, it also binds two kinases (interleukin receptor-associated kinase 1 [IRAK-1] and IRAK-4). IRAK-4 activates IRAK-1, and these in turn recruit tumor necrosis factor receptor-associated factor 6 (TRAF6). TRAF6 and other proteins then activate the IKK complex. Activation of IKK phosphorylates IkB leading to its destruction and the release of active NF-κB. The NF-κB in turn activates genes that encode the cytokines, IL-1, IL-18, and IL-33 as well as the type I interferons. IL-1 activation of NF-κB shares the same signaling pathways as TLR.

6.9.1.2

The NF-AT Pathway

When an antigen binds to its receptor on a T cell, the receptor (TCR) signals to the T cell. The signal is first transmitted from the antigen-binding TCR to a signal transducing complex called CD3, where it causes the CD3 chains to cluster together in lipid rafts (Figure 6-11). The CD3 proteins have specific amino acid sequences in their cytoplasmic domains called "immunoreceptor tyrosine-based, activation motifs" (ITAMs). When the chains cluster as an immunological synapse is formed, these ITAMs become binding sites for several TKs. These TKs are members of the Src-kinase family. They include lck and fyn in T cells and NK cells, and lyn and fyn in B cells and mast cells. In T cells, the first TK activated, called lck, phosphorylates the ITAMs. As a result these sites can bind a second TK, called zeta-associated protein-70 (ZAP-70). The bound ZAP-70 is phosphorylated in turn and then can trigger three signaling pathways. Via the second messengers, diacylglycerol and inositol trisphosphate, one pathway leads to the activation of the NF-AT. The inositol trisphosphate also releases calcium ions from intracel

78

FIGURE 6-11 Signal transduction mediated through T cell antigen receptors generates three transcription factors: nuclear factor of activated T cells *(NF-AT)*, nuclear factor kappa-B *(NF-κB)*, and activator protein-1 *(AP-1)*. Once T cell antigen receptors cluster they activate several protein kinases. The most important of these is called zeta-associated protein-70. This in turn triggers three signaling pathways and, with appropriate co-stimulation, generates multiple transduction factors. The jun-fos heterodimer (AP-1) is required to stimulate the genes for cytokines and their receptors. The final results of the stimulus include cell division or apoptosis as well as cytokine production.

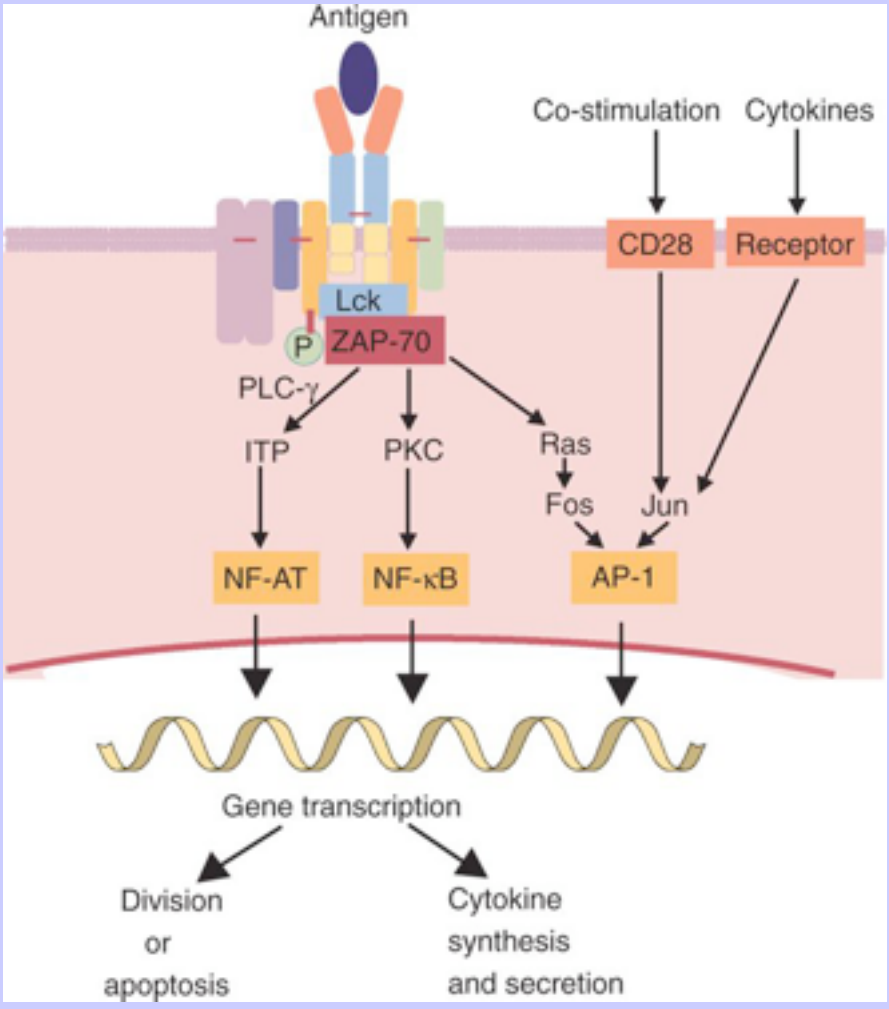
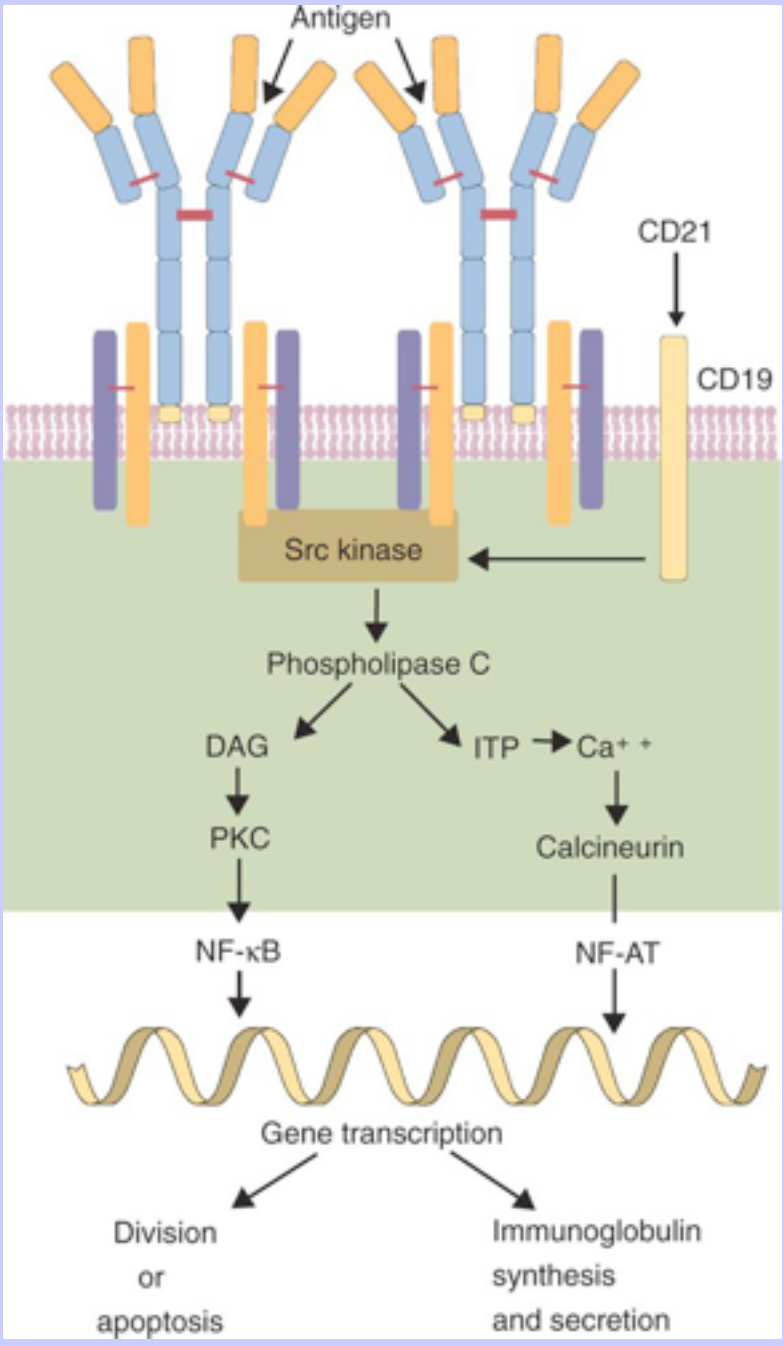


FIGURE 6-12 Signal transduction by two cross-linked B cell receptors activates B cells triggering cell division, differentiation, and immunoglobulin synthesis. Both nuclear factor kappa-B (NF-κB) and nuclear factor of activated T cells (NF-AT) are involved in B cell signal transduction.



lular organelles and opens transmembrane channels allowing Ca^{2+} to enter the cell and raising intracellular calcium. This in turn activates a phosphatase called calcineurin, which removes a phosphate from NF-AT. Dephosphorylated NF-AT enters the nucleus and with the help of another transcription factor, activator protein-1 (AP-1), binds to the promoters of at least 100 genes expressed in activated T cells. The potent immunosuppressive drugs tacrolimus and cyclosporine bind to calcineurin and so can block T cell–mediated responses.

In B cells, the adaptor molecules Iga and Igb have ITAMs. When aggregated by antigen and co-stimulated by CD19, the Src kinases lyn and fyn are activated. These in turn activate phospholipase C and eventually generate NF-κB and NF-AT (Figure 6-12).

The second pathway triggered by ZAP-70 activates a protein kinase C, which phosphorylates IkB and so activates NF-κB. The third pathway activates ras, a GTP-binding protein, that in turn activates fos. At the same time, co-stimulatory signals initiated by another receptor called CD28 lead to the activation of a protein called jun. The fos and jun proteins bind to form AP-1. AP-1 together with NF-AT activates multiple genes. The net effect of these reactions is that T cells enlarge, enter the cell cycle, and synthesize and secrete a mixture of cytokines. These cytokines trigger the next stages of the immune responses.

If the T cell receives other signals, such as those provided by IL-10 or TGF-β, the NF-AT may associate with a different transcription factor called Foxp3. This signal activates a very different set of genes and so converts the cell into a regulatory T cell (T_{reg}) that suppresses immune responses (see Chapter 17).

6.9.1.3 The JAK-STAT Pathway

The group I cytokine receptors signal through the JAK-STAT signaling pathway. The receptors for the JAK-STAT pathway are two identical single-pass transmembrane proteins. Each of their cytoplasmic ends binds a molecule of a JAK. Almost 40 cytokines use the JAK-STAT pathways including interleukins such as IL-2, IL-7, and IL-11 to IL-13, leptin GM-CSF and IFN-γ. Ligand binding to the receptor causes dimerization that leads to activation of the TK activity of the tightly associated JAK (Janus family TKs). These activated JAK molecules phosphorylate tyrosine residues on one of several STAT proteins (signal transducers and activators of transcription). The phosphorylated STAT proteins then dimerize, dissociate from JAK, and move to the nucleus, where they act as transcription factors and modulate the expression of target genes. There are four JAK and seven STAT family members recognized. A specific JAK-STAT combination is paired with each cytokine receptor. For example, receptors for the growth factors usually use JAK2. Receptors with the common γ chain preferentially use JAK1 and JAK3. The IFN-γ receptor uses JAK 1 and JAK2 (see Chapter 23, Figure 23-4). The IL-4R uses JAK1 and JAK3. Presumably the outcome of this signaling depends on which combination of JAK and STAT is activated.

78
79

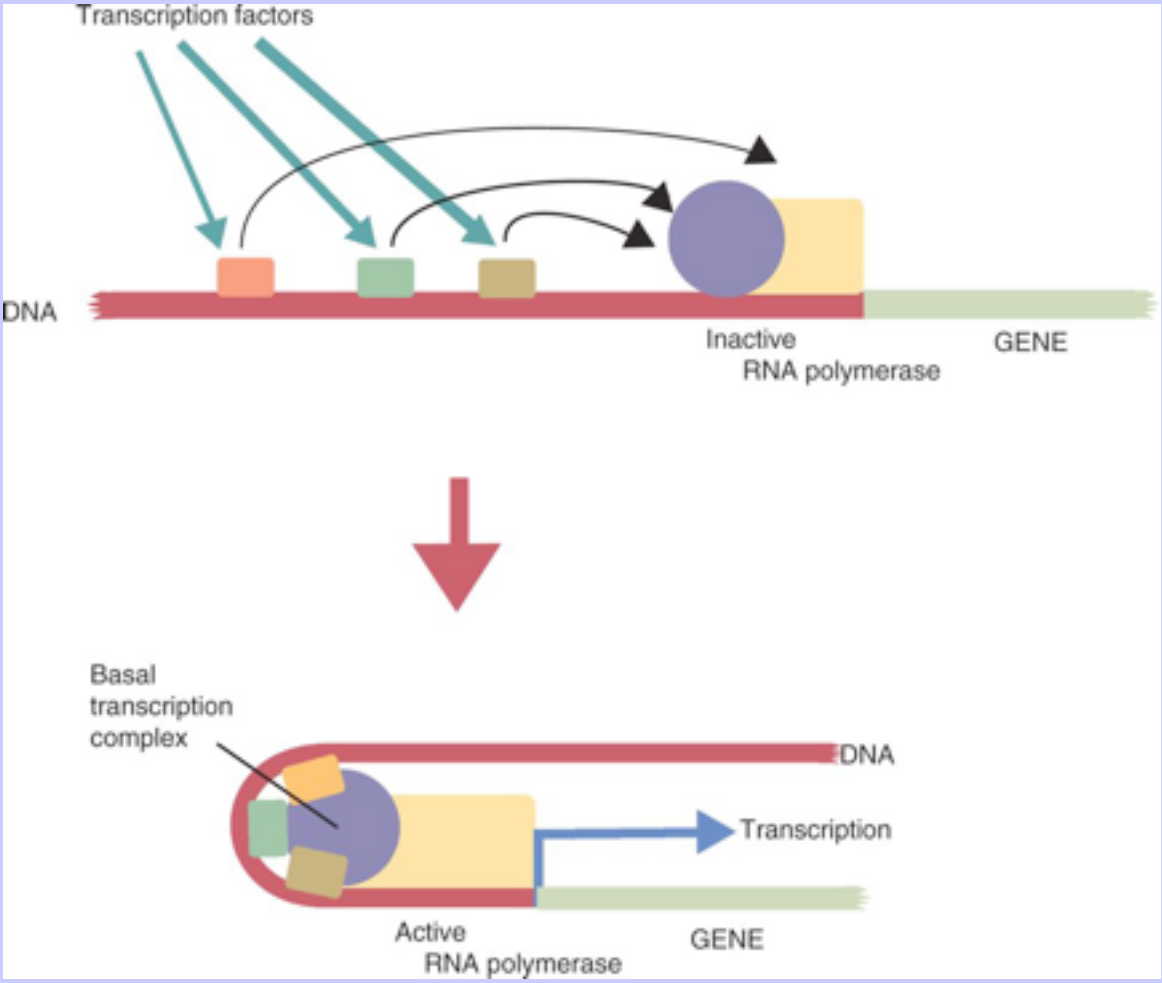
6.10 GENE TRANSCRIPTION

The activity of each gene in a cell is carefully controlled by many different mechanisms. Central to gene control are the transcription factors. Activation of genes depends on the presence of an appropriate mixture of transcription factors. As described above, these transcription factors are only generated when a cell receives an appropriate signal. The transcription factors then collectively activate the appropriate RNA polymerase (Figure 6-13).

Transcription factors have two binding sites. One site binds DNA; the other is a binding site for other proteins. When a transcription factor is activated, it enters the nucleus and binds to specific DNA control elements located between 50 and 200 bases upstream from the start site of the gene. Transcription factors may also bind to enhancer

elements located thousands of bases upstream. These bound transcription factors then bind either directly to basal transcription complexes or to a coactivator molecule. This binding leads to assembly of the basal transcription complex. The basal transcription complex together with any

FIGURE 6-13 Transcription factors bind to enhancer elements on DNA located upstream of the genes they activate. Gene transcription is turned on by a carefully regulated RNA polymerase. However, the polymerase can only be turned on when transcription factors activate the basic transcriptional machinery.



79

80

coactivator molecules then bind to the RNA polymerase and activate it. In the process it is believed that the conformation of the polymerase changes, activating it and permitting RNA transcription of the selected genes to begin.

6.11 SOURCES OF ADDITIONAL INFORMATION

H Fickenscher, S Hor, H Kupers, et al.: The interleukin-10 family of cytokines. *Trends Immunol*. **23**, 2002, 89–96.

ML Heaney, DW Golde: Soluble cytokine receptors. *Blood*. **87**, 1996, 847–857.

S Hurst, T Muchamuel, D Gorman, et al.: New IL-17 family members promote Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25. *J Immunol*. **169**, 2002, 443–453.

M Huse, BF Lillemeier, MS Zkuns, et al.: T cells use two directionally distinct pathways for cytokine secretion. *Nat Immunol*. **7**, 2006, 247–252.

D Metcalf: The hemopoietic regulators—an embarrassment of riches. *Bioessays*. **14**, 1992, 799–805.

In MJ Myers, MP Murtaugh (Eds.): *Cytokines in animal health and disease*. 1995, Marcel Dekker, New York.

B Nardelli, L Zaritskaya, M Semenuk, et al.: Regulatory effects of IFN-kappa, a novel type I IFN, on cytokine production by cells of the innate immune system. *J Immunol*. **169**, 2002, 4822–4830.

F Okano, M Satoh, T Ido, K Yamada: Cloning of cDNA for canine interleukin-18 and canine interleukin-1m converting enzyme and expression of canine interleukin-18. *J Interferon Cytokine Res*. **19**, 1999, 27–32.

C Parham, M Chirica, J Timans, et al.: A receptor for the heterodimeric cytokine IL-23 is composed of IL-12m1 and a novel cytokine receptor subunit, IL-23R. *J Immunol*. **168**, 2002, 5699–5708.

S Pflanz, JC Timans, J Cheung, et al.: IL-27, a heterodimeric cytokine composed of EB13 and p28 protein, induces proliferation of naive CD4+ T cells. *Immunity*. **16**, 2002, 779–790.

J Vilcek: Novel interferons. *Nat Immunol*. **4**, 2003, 8–9.

C Wietek, LAJ O'Neill: Diversity and regulation in the NF-kB system. *Trends Biochem Sci*. **32**, 2007, 301–309.

K Wolk, S Kunz, K Asadullah, R Sabat: Immune cells as sources and targets of the IL-10 family members. *J Immunol*. **168**, 2002, 5397–5402.